

Fluorescence Assay of of Ligands for Steroid Receptors in Living cells

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Steroid hormones regulate important biological events including reproduction, development, differentiation, homeostasis, and behavior. The activities of these hormones are mediated by their cognate steroid receptors (SRs) such as estrogen and androgen receptors (ER and AR, respectively). Several environmental/industrial chemicals having little structural resemblance with the natural hormones disrupt the endocrine system of a living body by mimicking or blocking the natural hormone activities by binding with the SR(s). These chemicals are called endocrine disruptors (EDs). An agonist binding to a SR induces a conformational change in the receptor ligand-binding domain (LBD), which promotes recruitment of a coactivator protein, and this interaction stimulates transcriptional activity. By contrast, an antagonist induces a different conformational change in the receptor LBD, which is not favorable for coactivator recruitment. Based ligand-induced coactivator recruitment to a SR, genetically encoded fluorescent indicators were developed to visualize the activities of estrogens and androgens in the physiological environment of single living cells. An ER LBD was connected to the steroid receptor coactivator-1 peptide via a flexible linker sequence. This fusion protein was sandwiched between cyan fluorescent protein (CFP, a donor) and yellow fluorescent protein (YFP, an acceptor) in such a way that excitation and emission spectra these fluorescent proteins are suitable for fluorescence resonance energy transfer from CFP to YFP. The indicator was named ER-*SCCoR* (single cell-coactivator recruitment), and the assay was called the fluorescence *SCCoR* assay. Replacing the ER LBD of the ER-*SCCoR* with AR LBD developed *SCCoR* variant for the AR, AR-*SCCoR*. An agonists addition to live cells expressed with *SCCoR* resulted in an increase in the FRET response. In contrast, no increase in FRET was observed with antagonist addition. The high sensitivity of the *SCCoR* indicators made it possible to distinguish between strong and weak estrogens and androgens in real-time in intact single living cells. The indicators can classify EDs as agonist or antagonists, rapidly and conveniently, within a few minutes upon addition of a ligand to live cells expressed with *SCCoR*. The SRs are important molecular targets for the treatment of several diseases including breast and prostate cancers. Our indicators can play an important role in the development of medicinal drugs that act through the ER or AR.

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